PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	16 JUN 2006
Applicant's or agent's file reference 157405.2 SB	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL04/01172	29 December 2004 (29.12.2004)	29 December 2003 (29.12.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC: G01N 33/53(2006.01),33/533(2006.01),33/534(2006.01),33/535(2006.01)
USPC: 435/7.1,7.91,7.94

Applicant

SAGI-EISENBERG

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	13 June 2006 (13.06.2006)	Unsu Jung Telephone No. 571-272-1600

Form PCT/ISA/237 (cover sheet) (April 2005)

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Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

   See the lack of unity section of the International Search Report(Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-19

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-19	YES
	Claims NONE	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-19	NO
Industrial applicability (IA)	Claims 1-19	YES
	Claims NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

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Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claim 1 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: the term "PKBP12" in lines 3 and 4 should be corrected to "FKBP12" as "FKBP12" is the correct term for FK506 binding protein.

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 1-4, 7-9, and 13-17 lack an inventive step under PCT Article 33(3) as being obvious over MacFarlane et al. (U.S. Patent No. 5,650,288, 22 July 1997) in view of Chen et al. (U.S. Patent No. 4,385,126, 24 May 1983) and Clackson et al. (U.S. Patent No. 6,187,757, 13 Feb 2001).

MacFarlane et al. (U.S. Patent No. 5,650,288, 22 July 1997) teaches that a major concern in immunosuppressive therapy associated with organ transplantation is the level of immunosuppressive drug circulating in the blood (column 1, lines 40-42). Toxicity can result if the immunosuppressive drug level is too high; graft rejection and opportunistic infection can result if the immunosuppressive drug level is too low (column 1, lines 42-45). MacFarlane et al. provides a method of assaying a sample of blood or blood components for the concentration of immunophilin ligand such as rapamycin using a variety of binding assays including receptor binding assay (column 2, lines 6-29). However, MacFarlane et al. fails to teach a method, wherein the receptor binding assays includes a sandwich format using immobilized FKBP12 and mTOR as a detecting receptor.

Chen et al. (U.S. Patent No. 4,385,126, 24 May 1983) teaches a well known method of sandwich assay, in which an assay ligand binds to an immobilized receptor ligand to form a first complex (column 1, lines 40-42). A tagged test ligand is then bound to the assay ligand in the complex to form the sandwich and the tagging constituent in the sandwiched ligands is detected quantitatively to deduce the quantity of assay ligand present. The detection can be performed by measuring radioactive, fluorescent, or enzyme labels present on the test ligand (column 1, lines 42-47). Furthermore, the quantity of assay ligand is deduced from quantity of test ligand detected using known standards (column 1, lines 58-62).

Clackson et al. (U.S. Patent No. 6,187,757, 13 Feb 2001) teaches that rapamycin binds to a FK506-binding protein (FKBP12, column 4, lines 57-62) with high affinity to form a rapamycin:FKBP complex, which binds with high affinity to the large cellular protein FRAP (mTOR, column 7, lines 56-67) to form FKBP:rapamycin:FRAP complex (column 1, lines 13-21). A number of rapamycin variants have been synthetically produced to improve the compound's therapeutic index as an immunosuppressive agent (column 2, lines 4-7).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to utilize a sandwich assay method of Chen et al., which employs two receptors for a detection ligand using a standard curve, in the method of assaying a blood sample for immunosuppressive drug, rapamycin, as taught by MacFarlane et al. et al. in order to perform an assay to detect concentration of rapamycin using known protein receptors of rapamycin, FKBP12 and mTOR as taught by Clackson et al. Furthermore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use FKBP12 as the immobilizing receptor since FKBP12

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binds to the rapamycin independent of mTOR, while mTOR binds to the FKBP12-rapamycin complex as taught by Clackson et al. The advantage of using the sandwich assay method, which is well known in the bind assay art to be one of the most sensitive assays for detecting target analytes in the sample, provides the motivation for combining the methods of Chen et al., Clackson et al., and MacFarlane et al. as the two rapamycin binding proteins, FKBP12 and mTOR, have high affinity for rapamycin with a reasonable expectation of success since MacFarland teaches that a variety of binding assays including receptor binding assay can be used to determine level of immunosuppressive drug circulating in the blood.

With respect to claim 2, Clackson et al. teaches that a variety of rapamycin analogs (synthetic) are used as a therapeutic agent (column 2, lines 4-22) and also bind to FKBP12 (column 4, lines 57-62) and mTOR (column 7, lines 31-67). Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to determine the concentration of rapamycin or rapamycin analog in patients in order to determine rapamycin analog levels in patients receiving rapamycin analogs as FKBP12 and mTOR form FKBP:rapamycin:FRAP complex in the presence of rapamycin or rapamycin analogs.

With respect to claims 3, 4, and 7, MacFarland et al. teaches a sample, which is a clinical blood sample (column 1, lines 14-45).

With respect to claims 14 and 16, Chen et al. teaches that the test ligand (FRB fragment) is directly bound to a detectable label, which can be detected by spectrophotometry, fluorospectrophotometry, and radiospectrometry (column 1, lines 42-47).

With respect to claims 15 and 17, Chen et al. teaches that the test ligand (FRB fragment) is indirectly bound to a detectable a detectable label (enzyme, column 1, lines 42-47).

Claims 5 and 6 lack an inventive step under PCT Article 33(3) as being obvious over MacFarlane et al. (U.S. Patent No. 5,650,288, 22 July 1997) in view of Chen et al. (U.S. Patent No. 4,385,126, 24 May 1983) and Clackson et al. (U.S. Patent No. 6,187,757, 13 Feb 2001) as applied to claim 3 above, and further in view of Ormerod et al. (WO 99/24036, 20 May 1999).

MacFarland et al. in view of Chen et al. and Clackson et al. teaches an assay for determining rapamycin or rapamycin analog concentrations in a sample as discussed above. However, MacFarland et al. in view of Chen et al. and Clackson et al. fails to teach a method, wherein the sample is a solid sample selected from tissues or feces.

Ormerod et al. teaches a method of using sirolimus (rapamycin) for the treatment of dermatological condition, in which a therapeutic amount of rapamycin is applied to the skin with a minimal systemic effect (Abstract). Ormerod et al. further teaches that tissue concentrations of rapamycin are used in order to optimize the therapeutic rapamycin concentration and the ratio of permeation enhancer and solvent (p9, lines 4-29).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include skin tissue as a sample in the assay of MacFarland et al. in view of Chen et al. and Clackson et al. as taught by Ormerod et al. in order to determine tissue concentrations of rapamycin, which is used to optimize the therapeutic rapamycin concentration and the ratio of permeation enhancer and solvent. The advantage of determining tissue concentrations of rapamycin, which is used to optimize the therapeutic rapamycin concentration and the ratio of permeation enhancer and solvent provides the motivation to combine the methods of MacFarland et al., Chen et al. and Clackson et al., and Ormerod et al. with a reasonable expectation of success as the method of MacFarland et al. of using nonionic detergent and a protease would lyse cellular membranes to obtain rapamycin concentration (column 2, lines 6-14).

Claims 10-12, 18, and 19 lack an inventive step under PCT Article 33(3) as being obvious over MacFarlane et al. (U.S. Patent No. 5,650,288, 22 July 1997) in view of Chen et al. (U.S. Patent No. 4,385,126, 24 May 1983) and Clackson et al. (U.S. Patent No. 6,187,757, 13 Feb 2001) as applied to claim 3 above, and further in view of Coligan et al. (Current Protocols in Immunology, Vol. 1, 1991, John Wiley & Sons, Inc., pp2.1.1-2.1.22).

MacFarland et al. in view of Chen et al. and Clackson et al. teaches an assay for determining rapamycin or rapamycin analog concentrations in a sample as discussed above. However, MacFarland et al. in view of Chen et al. and Clackson et al. fails to teach a method, wherein the solid support is a 96-well microtiter plate, which is blocked by non specific protein and the detection is achieved by an ELISA reader.

Coligan et al. teaches methods of variety of sandwich assays, which are typically conducted on a 96-well microtiter plates and read by an ELISA reader (p2.1.17, Fig. 2.1.7). Coligan et al. further teaches a blocking step, which blocks residual binding capacity of the plate following immobilization of capture antibody (p2.1.5, *Block residual binding capacity of plate*). Coligan teaches a well known enzyme conjugate label, alkaline phosphatase with p-nitrophenyl phosphate (NPP, p2.1.3, *Materials*) as a substrate as a color forming agent (p2.1.10, steps 9 and 11).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a 96 well microtiter plate, which is blocked by non specific protein and detected by an ELISA reader, wherein alkaline phosphatase is used as an enzyme label with p-nitrophenyl phosphate as a substrate as taught by Coligan et al. in the method of MacFarland et al. in view of Chen et al. and Clackson et al. in order to perform sandwich assay for determining rapamycin concentrations in a sample. The advantage conducting multiple

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sample analysis of rapamycin using 96-well microtiter plate with an ELISA reader, which is designed for reading 96-well plate, provides the motivation for combining the methods of MacFarland et al., Chen et al., Clackson et al., and Coligan et al. Furthermore, one of ordinary skill in the art at the time of the invention would have recognized that blocking step of Coligan et al. is a necessary step in a sandwich assay to block binding of non specific proteins. Therefore, the advantage of including a blocking step in order to block binding of non specific proteins provides the motivation for combining the methods of MacFarland et al., Chen et al., Clackson et al., and Coligan et al. as the blocking step would increase the specificity of the assay of MacFarland et al. in view of Chen et al. and Clackson et al. In addition, one of ordinary skill in the art would have found it obvious to employ the alkaline phosphatase is used as an enzyme label with p-nitrophenyl phosphate as a substrate as taught by Coligan et al. since it appears that any well know enzyme label and corresponding substrate such as alkaline phosphatase and p-nitrophenyl phosphate would perform equally well with the assay method of MacFarland et al. in view of Chen et al. and Clackson et al.

Claims 1-19 meet the criteria set out in PCT Article 33(4), and thus satisfies industrial applicability because the subject matter claimed can be made or used in industry.